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L8: Entry 21 of 32 File: USPT Nov 16, 1999

DOCUMENT-IDENTIFIER: US 5985846 A
TITLE: Gene therapy for muscular dystrophy

Detailed Description Text (61):
(c) adenovirus DNA, which has been freed from the virion capsid as in (b) with the terminal protein still attached to the terminus, but that has not been purified by gel filtration or sucrose centrifugation, rather such is directly used for transfection after lysing the adenovirus particles by incubation on ice for 20 min in a buffer comprising 8.0 M guanidinium hydrochloride, 10 mM Tris-HCl (pH 7.5), 2.0 mM EDTA and 4.0 mM .beta.-mercaptoethanol.

Detailed Description Text (67):
Harvesting of the adenovirus particles produced by the co-transfected host cells can be carried out by well-known methods, such as resuspension of the cells in phosphate buffered saline (PBS) containing 5-10% (v/v) glycerol, followed by preparation of a cell extract by freezing and thawing of the cells, as described by Mitani et al, supra.

Detailed Description Text (80):
After CsCl centrifugation, the recombinant adenovirus particles can be dialyzed against PBS or Tris-buffered saline to remove the CsCl, and stored at -20 to -80.degree. C. prior to use.

Detailed Description Text (189):
Southern blot analysis (Southern, J. Mol. Biol., 98:503 (1975) was carried out using the resulting gel and 5.0 ng of .sup.32 P-labeled pDYS.beta.gal DNA or Ad5 DNA as the probe. The probes were prepared as described by Feinberg et al, Anal. Biochem., 132:6 (1983). Pre-hybridization and hybridization were performed in 1.5.times. SSPE containing 1.0% (w/v) SDS, 1.0% (v/v) nonfat milk and 20% (w/v) dextran sulfate at 65.degree. C. for 12-16 hr. 20.times. SSPE comprises 3.0 M NaCl, 0.5 M NaH.sub.2 PO.sub.4 and 2.0 mM EDTA (pH 7.4). The membranes were washed twice for 15 min with 2.times. SSC containing 0.5% (w/v) SDS. 20.times. SSC comprises 3.0 M NaCl and 0.3 M Na-Citrate. Next, the membranes were washed three times for 20 min with 0.1.times. SSC containing 0.5% (w/v) SDS at 65.degree. C., followed by exposure to X-omat AR X-ray films for 1-12 hr.

Detailed Description Text (204):
The monolayers were rinsed three times with PBS, and three times with buffer comprising 50 mM Tris-HCl (pH 8.0), 5.0 mM EDTA and 5.0 mM EGTA (hereinafter "TEE buffer"). The cells were collected in a small volume of TEE buffer, and centrifuged at 14,000.times.g for 5 min at 4.degree. C. The resulting cell pellet was resuspended in TEE buffer containing 0.3% (w/v) SDS, and incubated on ice for 20 min. Brief sonication was then carried out to reduced the viscosity of the sample. Total protein concentration was assayed using the bicinchoninic acid protein assay reagent (Pierce, Rockford, Ill.). 25 .mu.g of protein from the sonicated cell pellet were separated by electrophoresis on a 5.0% (w/v) SDS-PAGE gel, and transferred to a nitrocellulose membrane. The membrane was blocked with 5.0% (v/v) nonfat milk and 5.0% (v/v) goat serum in Tris-buffered saline-Tween comprising 20 mM Tris-HCl (pH 8.0), 137 mM NaCl and 0.1% (v/v) polyoxyethylene sorbitan monolaurate (Tween 20) (hereinafter "TBS-T") for 1 hr. Then, immunostaining was

performed according to the protocol for ECL Western blotting detection reagents (Amersham Life Science, Buckinghamshire, UK) using DYS2 (Novocastra Laboratories, Newcastle upon Tyne, UK), a mouse monoclonal antibody directed against the carboxy terminal 17 amino acids of dystrophin, diluted 1:100 in TBS-T, as the primary antibody, and horseradish peroxidase-conjugated anti-mouse antibody (Kirkegaard & Perry Laboratories, Gaithersburg, Md.), diluted 1:5000 in TBS-T as the secondary antibody. The chemiluminescent signal was detected by a 30 sec exposure to autoradiography film.

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L20: Entry 3 of 15 File: PGPB Nov 13, 2003

DOCUMENT-IDENTIFIER: US 20030211598 A1
TITLE: Compositions and methods for therapeutic use

Summary of Invention Paragraph:
[0012] A further aspect of the invention is a pharmaceutical formulation for administration of a recombinant adenovirus, comprising about 10.sup.9-10.sup.11 particles (PN)/ml recombinant adenovirus, about 2-10 mM Big CHAP or about 0.1-1.0 mM TRITON.RTM.-X-100 detergent, phosphate buffered saline (PBS), about 2-3% sucrose (w/v) and about 1-3 mM MgCl.sub.2, about pH 6.4-8.4.

Detail Description Paragraph:
[0031] The buffer containing the delivery-enhancing agent may be any pharmaceutical buffer such as phosphate buffered saline or sodium phosphate/sodium sulfate, Tris buffer, glycine buffer, sterile water and other buffers known to the ordinarily skilled artisan-such as those described by Good et al. (1966) Biochemistry 5:467. The pH of the buffer in the pharmaceutical composition comprising the tumor suppressor gene contained in the adenoviral vector delivery system, may be in the range of 6.4 to 8.4, preferably 7 to 7.5, and most preferably 7.2 to 7.4.

Detail Description Paragraph:
[0032] A preferred formulation for administration of a recombinant adenovirus is about 10.sup.9-10.sup.11 PN/ml virus, about 2-10 mM Big CHAP or about 0.1-1.0 mM TRITON.RTM.-X-100 detergent, in phosphate buffered saline (PBS), plus about 2-3% sucrose (w/v) and about 1-3 mM MgCl.sub.2, at about pH 6.4-8.4.

CLAIMS:

39. A pharmaceutical formulation for administration of a recombinant adenovirus, comprising about 10.sup.9-10.sup.11 PN/ml recombinant adenovirus, about 2-10 mM Big CHAP or about 0.1-1.0 mM TRITON.RTM.-X-100 detergent, phosphate buffered saline (PBS), about 2-3% sucrose (w/v) and about 1-3 mM MgCl.sub.2, about pH 6.4-8.4.